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Thermal Dissociation and Conformational Lock of Superoxide Dismutase

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The kinetics of thermal dissociation of superoxide dismutase (SOD) was studied in 0.05 M Tris-HCl buffer at pH 7.4 containing 10^{-4} M EDTA. The number of conformational locks and contact areas and amino acid residues of dimers of SOD were obtained by kinetic analysis and biochemical calculation. The cleavage bonds between dimers of SOD during thermal dissociation and type of interactions between specific amino acid residues were also simulated. Two identical contact areas between two subunits were identified. Cleavage of these contact areas resulted in dissociation of the subunits, with destruction of the active centers, and thus, lost of activity. It is suggested that the contact areas interact with active centers by conformational changes involving secondary structural elements.

Keywords: Biochemical calculation, Conformational lock, Contact area, Kinetics, Superoxide dismutase (SOD), Thermal dissociation

Introduction

Superoxide dismutases (Cu, Zn-SODs) have been found in the cytoplasm of all the eukaryotic cells and in the periplasm of several bacterial species (Bannister *et al*., 1987; Kroll *et al*., 1995). Eukaryotic Cu, Zn-SODs are homodimers that contain one atom of zinc and one atom of copper per subunit and catalyze the dismutation of the superoxide anion at a diffusion-limited rate enhanced by electrostatic guidance of the substrate to the active site (Desideri *et al*., 1992). Cu, Zn-SODs possess a very compact structure that is highly resistant to denaturation by urea, SDS and proteolytic degradation. Several factors are thought to contribute to the enzyme stability, including the prosthetic metal ions (Forman and Fridovich, 1973), the intrasubunit disulfide bond (Abernethy *et al*., 1974), and the close packing of the hydrophobic interface between the subunits and the two halves of the β-barrel core (Getzoff *et al*., 1989). Amino acid sequence comparisons and the analysis of the three dimensional structure of the dimeric enzyme from *P.leiognathi* have shown that the prokaryotic and eukaryotic Cu, Zn-SODs share a conserved ligand stereochemistry and a very similar monomer fold, based on a flattened Greek-key eight-stranded β-barrel (Bordo *et al*., 1994; Bourne *et al*., 1996; Imlay and Imlay, 1996; Battistoni *et al*., 1996).

Since the discovery of Cu, Zn-SOD, there has been serious in understanding how the dimeric structure contributes to the high catalytic efficiency and the remarkable stability of this class of enzymes. All attempts to obtain monomeric Cu, Zn-SODs by treatments with detergents (Rigo *et al*., 1978) or site-directed substitutions of hydrophobic residues at the dimer interface (Bertini *et al*., 1994) provided also enzymes that display very low catalytic activity and gross alterations of spectroscopic properties. These dramatic changes probably reflect changes in the tertiary structure consequent to rearrangements of the solvent-exposed hydrophobic dimer interface.

The properties of interfaces in oligomeric enzymes and their influence on catalytic activity can be studied and explained by two independent methods, involving the use of the structural and kinetics data. Poltorak *et al*. (1999a; 1999b) reported that, in alkaline phosphatase from different sources, the same results that in both methods are in reasonable agreement. In our previous paper (Moosavi-Nejad *et al*., 2003), we had put forward a mechanism of Lentil seedling amine oxidase denaturation based on structural data. In

Abbreviations: SOD, superoxide dismutase; T_{opt}, optimum temperature; EDTA, ethylenediaminetetracetic acid; Tris, tris(hydroxymethyl) aminomethane; SDS, sodium dodecyl sulfate

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